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BIOASSAY OF 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE FOR POSSIBLE CARCINOGENICITY

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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REPORT ON THE BIOASSAY OF 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

FOREWORD: This report presents the results of the bioassay of 2-(chloromethyl)pyridine hydrochloride conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of 2-(chloromethyl)pyridine hydrochloride was conducted by Litton Bionetics, Inc., Kensington, Maryland, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. N. P. Page (1,2), Dr. E. K. Weisburger (1) and Dr. J. H. Weisburger (1,3). The principal investigators for the contract were Dr. F. M. Garner (4) and Dr. B. M. Ulland (4,5). Mr. S. Johnson (4) was the coprincipal investigator for the contract. Animal treatment and observation were supervised by Mr. R. Cypher (4), Mr. D. S. Howard (4) and Mr. H. D. Thornett (4); Mr. H. Paulin (4) analyzed dosed feed mixtures. Ms. J. Blalock (4) was responsible for data collection and assembly. Chemical analysis was performed by Midwest Research Institute (6) and the analytical results were reviewed by Dr. N. Zimmerman (7).

Histopathologic examinations were performed by Drs. A. DePaoli (4), P. Hildebrandt (4), R. Montali (4), C. Montgomery (4), H. Seibold (4), N. Wosu (4), and B. Zook (4) and reviewed by Dr. A. DePaoli (4), at Litton Bionetics, Inc., the pathology narratives were written by Dr. A. DePaoli (4), and the diagnoses included in this

report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (8).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (9); the statistical analysis was performed by Mr. R. M. Helfand (7) and Dr. J. P. Dirkse, III (10) using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (11).

This report was prepared at METREK, a Division of The MITRE Corporation (7) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (7), task leader Ms. P. Walker (7), senior biologist Mr. M. Morse (7), biochemist Mr. S. C. Drill (7), and technical editor Ms. P. A. Miller (7). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. R. A. Griesemer (1), Dr. T. E. Hamm (1), Dr. W. V. Hartwell (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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SUMMARY

A bioassay for the possible carcinogenicity of 2-(chloromethyl) pyridine hydrochloride was conducted using Fischer 344 rats and B6C3F1 mice. 2-(Chloromethyl)pyridine hydrochloride was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species, with the exception of 49 male rats in the high dose group. Twenty animals of each sex and species were placed on test as vehicle controls. The high and low dosages of 2-(chloromethyl)pyridine hydrochloride administered were, respectively, 150 and 75 mg/kg for rats and 250 and 125 mg/kg for mice. The compound was administered for 99 weeks to rats and mice. The period of compound administration was followed by an observation period of 6 weeks for rats and 5 weeks for mice.

There were no significant positive associations between the dosages of 2-(chloromethyl)pyridine hydrochloride administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Slight dose-related mean body weight depression was observed in mice of both sexes, indicating that the dosages of 2-(chloromethyl)pyridine hydrochloride administered to these animals in this bioassay may have approximated the maximum tolerated concentrations. Since no distinct mean body weight depression relative to vehicle controls, no significant accelerated mortality, and no other signs of toxicity were associated with administration of 2-(chloromethyl)pyridine hydrochloride to rats, it is possible that these animals may have been able to tolerate a higher dosage.

None of the statistical tests for any site in female rats or in mice of either sex indicated a significant positive association between compound administration and tumor incidence. There was a significant positive trend between the dosages administered and the incidences of subcutaneous fibromas in male rats. The Fisher exact comparisons, however, were not significant.

Under the conditions of this bioassay, administration of 2-(chloromethyl)pyridine hydrochloride was not carcinogenic to Fischer 344 rats or B6C3F1 mice.

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I. INTRODUCTION

2-(Chloromethyl)pyridine hydrochloride (Figure 1) (NCI No. C03907), an aromatic heterocycle used in a variety of syntheses, was selected for bioassay by the National Cancer Institute because of the structural similarity of this compound to 2-(α,β-dichloroethyl)-pyridine hydrochloride, a carcinogen in rats, mice, Syrian hamsters, and Mongolian gerbils (Harris, 1968).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 2-(chloromethyl)pyridine hydrochloride.* It is also called 2-(Cl-methyl)pyridine HCl; 2-pyridylmethyl chloride hydrochloride; and 2-picolyl chloride hydrochloride.

2-(Chloromethyl)pyridine hydrochloride has been used to synthesize a variety of compounds, such as pyridylalkyl-(2-anilinophenyl) acetates, useful as uv absorbers for antisunburn creams, analgesics, and anti-inflammatory agents (Haas and Sallmann, 1974); 5,5-disubstituted barbituric acids (Stevens et al., 1973b); and S-(pyridylmethyl) thiocarbamates, which possess herbicidal activity against hairy crabgrass, watergrass, California red oats, curly dock, and several other weeds (Tilles and Brokke, 1972). This compound has also been found to be nematocidal, preventing the development of root knots on tomato seedlings (Fuhlhage, 1970).

*The CAS registry number is 6959-47-3

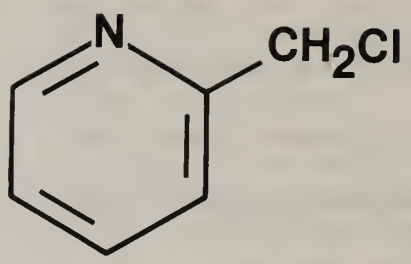


FIGURE 1
CHEMICAL STRUCTURE OF 2-(CHLOROMETHYL)PYRIDINE (HYDROCHLORIDE)

2-(Chloromethyl)pyridine has also been used as an intermediate in the preparation of various compounds, such as substituted anilinophenylacetic-acid-(2-pyridyl)-methyl esters and derivatives, which possess analgesic and anti-inflammatory activity and can be used as uv absorbers for cosmetics (Haas and Sallmann, 1975); hypocholesteremic and analgesic piperazine derivatives (Nakanishi et al., 1973); barbituric acid derivatives (Stevens et al., 1973a); and 1-methyl-2-pyridones (Matsumura et al., 1970).

Specific production data for 2-(chloromethyl)pyridine hydrochloride and 2-(chloromethyl)pyridine are not available; however, neither of these compounds is produced in commercial quantities (in excess of 1000 pounds or $1000 in value annually) in the United States (U.S. International Trade Commission, 1977).

These compounds apparently do not have any large-scale uses; however, the attention devoted to them by pharmaceutical researchers suggests that 2-(chloromethyl)pyridine or its hydrochloride salt may be used to prepare currently used drugs in small but significant quantities. Thus, the potential for exposure may not be restricted to researchers, but may also exist for a limited number of workers in the pharmaceutical manufacturing industry.

II. MATERIALS AND METHODS

A. Chemicals

Three batches of technical-grade 2-(chloromethyl)pyridine hydrochloride were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. Chemical analysis was performed by Midwest Research Institute, Kansas City, Missouri. The experimentally determined range in melting point of the first and second batches, 119° to 127°C and 124° to 126°C, respectively, were compared to the literature value of 128° to 129°C for the standard material (Mathes and Schuely, 1963). No melting point was reported for the third batch. The results of thin-layer chromatography were similar for the first and second batches (i.e., one major spot and one minor spot were visualized). The result for the third batch (i.e., either two or three impurities) deviated from those produced by analysis of the first two batches;. however, different solvent systems were utilized. Ultraviolet/visible analysis for the first and second batches indicated λ_{max} at 261 nm with a molar extinction coefficient (ϵ) of 4×10^3. For the third batch, analysis revealed λ_{max} at 262 nm with ϵ of 4.4×10^3. The literature value (Sadtler Standard Spectra) indicated λ_{max} at 261 nm with ϵ of 31×10^2.

Throughout this report, the term 2-(chloromethyl)pyridine HCl is used to represent this technical-grade material.

B. Dosage Preparation

Fresh solutions of 2-(chloromethyl)pyridine HCl in distilled water (Borden Polar Water Company, Beltsville, Maryland) were prepared on each day that intubation was performed. Excess portions of the mixtures were disposed of rather than stored. The concentration of 2-(chloromethyl)pyridine HCl in distilled water ranged from 0.75 to 1.5 percent for rats and from 1.25 to 2.5 percent for mice.

Dosed distilled water preparations containing 5359 and 7432 ppm of 2-(chloromethyl)pyridine HCl were analyzed spectrophotometrically. The mean result immediately after preparation was 92 percent of theoretical (ranging from 87 to 99 percent).

C. Animals

The two animals species, Fischer 344 rats and B6C3F1 mice, used in the carcinogenicity bioassay were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. Rats were supplied by the Frederick Cancer Research Center, Frederick, Maryland. Mice were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

Rats and mice were approximately 4 weeks old when received. Upon receipt, animals were examined and any obviously ill or runted animals were killed. The remaining animals were quarantined for 2 weeks prior to initiation of test. Animals which did not manifest clinical signs of disease were placed on test at this time. Animals were assigned to

groups and distributed among cages so that the average body weight per cage was approximately equal for a given species and sex.

D. Animal Maintenance

Animals were housed by species in rooms with a temperature range of 22° to 26°C and a range in relative humidity of 45 to 55 percent. Incoming air was filtered through HEPA filters (Flanders Filters, McLean, Virginia) at a rate of 12 to 15 complete changes of room air per hour. Fluorescent lighting was provided 8 hours per day (9:00 a.m. to 5:00 p.m.).

Rats were housed four per cage by sex and mice were housed five per cage by sex. Throughout the study dosed and control animals of both species were housed in polycarbonate cages (Lab Products, Inc., Garfield, New Jersey) suspended from aluminum racks. Racks were fitted with a continuous piece of stainless steel mesh over which a sheet of filter paper was firmly secured. Filter paper was changed at 2-week intervals, when the racks were sanitized. Clean cages and bedding (Ab-sorb-dri® hardwood chip bedding [Wilner Wood Products Company, Norway, Maine]) were provided twice weekly.

Acidulated water (pH 2.5) was supplied to animals in water bottles which were changed and washed twice weekly. Sipper tubes were washed at weekly intervals. All animals were supplied with Wayne Lab-Blox® meal in hanging stainless steel hoppers which were refilled three times per week and sanitized weekly. Food and water were available ad libitum for both species.

All dosed and control rats were housed in a room with other rats receiving diets containing* 4-amino-2-nitrophenol (119-34-6) and p-phenylenediamine dihydrochloride (624-18-0).

All dosed and control mice were housed in a room with mice receiving diets containing 2,4-dimethoxyaniline hydrochloride (54150-69-5); 4'-(chloroacetyl)-acetanilide (140-49-8); p-phenylenediamine dihydrochloride (624-18-0); 4-nitro-o-phenylenediamine (99-56-9); and 1-phenyl-3-methyl-5-pyrazolone (89-25-8); and other mice intubated with trimethylphosphate (512-56-1); 3-(chloromethyl)pyridine hydrochloride (3099-31-8); and pivalolactone (1955-45-9).

E. Gastric Intubation

Intubation was performed three days per week on a mg/kg body weight basis, utilizing the most recently observed group mean body weight as a guide for determining the dose. All animals were weighed and dosages adjusted once monthly, based on group mean body weight. Thus, although the ratio of dose to weight remained constant, the total dosage administered fluctuated with an increase or decrease in group mean body weight. Animals of each sex within a dosed group received the same dosage.

F. Selection of Initial Dose Levels

To establish the dosages of 2-(chloromethyl)pyridine HCl for administration to dosed animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Animals of

*CAS registry numbers are given in parentheses.

each species were distributed among six groups, each consisting of five males and five females. 2-(Chloromethyl)pyridine HCl mixed with distilled water was introduced by gavage to five of the six rat groups at dosages of 68, 100, 147, 215 and 316 mg/kg and to five of the six mouse groups at dosages of 100, 147, 215, 316 and 464 mg/kg. The sixth group of each species served as a vehicle control, receiving only distilled water by gavage.

Intubation was performed three days per week for 7 weeks, followed by a 1-week observation period to detect any delayed toxicity. Individual body weights were recorded weekly throughout the study. Upon termination of the study all survivors were sacrificed and necropsied.

The following table indicates the mean body weight gain, relative to controls, and survival observed in each of the rat groups at the end of the subchronic test.

RAT SUBCHRONIC STUDY RESULTS

	Mean Body Weight Gain (%)*		Survival**	
mg/kg	Males	Females	Males	Females
316	-24	-6	5/5	2/5
215	-15	+3	5/5	5/5
147	- 5	+2	5/5	5/5
100	- 6	0	5/5	5/5
68	-13	+5	5/5	5/5
0	--	--	5/5	5/5

*+ is indicative of mean body weight gain greater than that of controls.
- is indicative of mean body weight gain less than that of controls.
**Number of animals observed/number of animals originally in group.

No other clinical abnormalities which could be attributed to administration of the compound were observed. The high dosage selected for administration to dosed rats in the chronic bioassay was 150 mg/kg.

The following table indicates the mean body weight gain, relative to controls, survival, and incidence of rough hair and arched backs observed in each of the mouse groups at the end of the subchronic test.

MOUSE SUBCHRONIC STUDY RESULTS

	Mean Body Weight Gain (%)*		Survival**		Observation of Rough Hair and Arched Backs**	
mg/kg	Males	Females	Males	Females	Males	Females
464	-2	-7	5/5	4/5	5/5	5/5
316	+5	0	5/5	5/5	0/5	0/5
215	+3	-3	5/5	5/5	0/5	0/5
147	+2	-3	5/5	5/5	0/5	0/5
100	+8	-1	5/5	5/5	0/5	0/5
0	--	--	5/5	5/5	0/5	0/5

The high dosage selected for administration to dosed mice in the chronic bioassay was 250 mg/kg.

G. Experimental Design

The experimental design parameters for the chronic study (species, sex, group size, dosages administered, and duration of treated and untreated observation periods) are summarized in Tables 1 and 2.

*+ is indicative of mean body weight gain greater than that of controls.
- is indicative of mean body weight gain less than that of controls.
**Number of animals observed/number of animals originally in group.

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
2-(CHLOROMETHYL)PYRIDINE HCl GAVAGE EXPERIMENT

	INITIAL GROUP SIZE	2-(CHLOROMETHYL) PYRIDINE HCl DOSAGE[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
VEHICLE CONTROL	20	0	0	105[b]
LOW DOSE	50	75	99	
		0		6
HIGH DOSE	49	150	99	
		0		6
FEMALE				
VEHICLE CONTROL	20	0	0	105[b]
LOW DOSE	50	75	99	
		0		6
HIGH DOSE	50	150	99	
		0		6

[a]Dosages, given in mg/kg body weight, were administered by gavage 3 days per week.

[b]Gavaged with distilled water 3 days per week for 99 weeks.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
2-(CHLOROMETHYL)PYRIDINE HCl GAVAGE EXPERIMENT

	INITIAL GROUP SIZE	2-(CHLOROMETHYL) PYRIDINE HCl DOSAGE[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
VEHICLE CONTROL	20	0	0	104[b]
LOW DOSE	50	125	99	
		0		5
HIGH DOSE	50	250	99	
		0		5
FEMALE				
VEHICLE CONTROL	20	0	0	104[b]
LOW DOSE	50	125	99	
		0		5
HIGH DOSE	50	250	99	
		0		5

[a]Dosages, given in mg/kg body weight, were administered by gavage 3 days per week.

[b]Gavaged with distilled water 3 days per week for 99 weeks.

All rats were approximately 6 weeks old at the time the test was initiated and were placed on test on the same day. Dosed rats were intubated with 150 and 75 mg/kg 2-(chloromethyl)pyridine HCl for 99 weeks followed by a 6-week observation period, when no test chemicals were used. Throughout this report those rats receiving the former dosage are referred to as the high dose groups and those receiving the latter dosage are referred to as the low dose groups.

All mice were approximately 6 weeks old at the time the test was initiated and were placed on test on the same day. Dosed mice were intubated with 250 and 125 mg/kg 2-(chloromethyl)pyridine HCl for 99 weeks followed by a 5-week observation period, when no test chemicals were used. Throughout this report those mice receiving the former dosage are referred to as the high dose groups and those receiving the latter dosage are referred to as the low dose groups.

Vehicle control animals were intubated with 10 ml/kg distilled water 3 days per week for the same period that dosed animals were intubated.

H. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment and body weights were recorded at monthly intervals throughout the bioassay. All animals were inspected twice daily. Food consumption data were collected at monthly intervals from 20 percent of the animals in each group.

All moribund animals or animals that developed large, palpable masses that jeopardized their health were killed. A necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was killed at the end of the bioassay. The animals were euthanized using carbon dioxide, and were immediately necropsied. Gross and microscopic examinations were performed on all major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in a 10 percent neutral buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination.

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, uterus, mammary gland, and ovary.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of

animals that were recorded in each group at the time that the test was initiated.

I. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when testing a dose-related trend. One-tailed P-values have been reported

for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site was examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It

is not, however, presented in the tables, where the Fisher exact P-values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971, pp. 362-365), was also used

is not, however, presented in the tables, where the Fisher exact P-values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971, pp. 362-365), was also used when appropriate. Under the assumption of a linear trend, this test determined if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses.

The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

Although vehicle control male rats did weigh slightly more than dosed male rats for a major portion of the bioassay, no dose-related mean body weight depression was apparent in either male or female rats (Figure 2).

No other clinical signs were recorded.

B. Survival

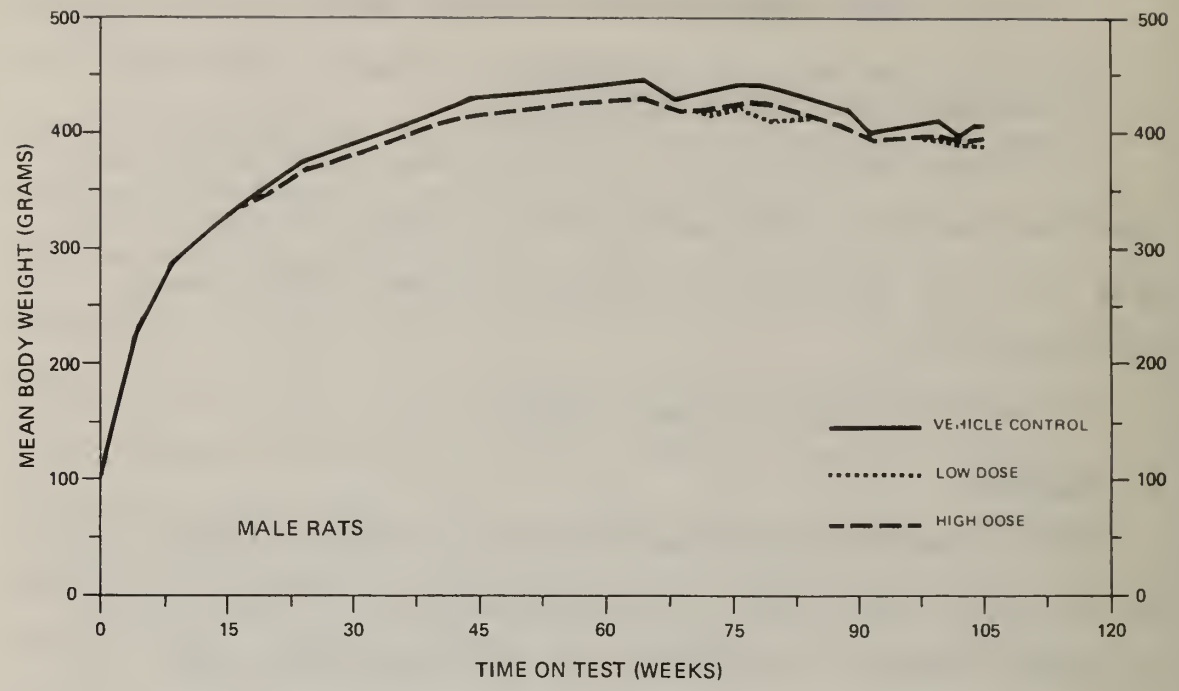
The estimated probabilities of survival for male and female rats in the vehicle control and 2-(chloromethyl)pyridine HCl-dosed groups are shown in Figure 3. The Tarone test for association between dosage and mortality was not significant for either males or females.

There were adequate numbers of male rats at risk from late-developing tumors as 67 percent (33/49) of the high dose, 80 percent (40/50) of the low dose, and 75 percent (15/20) of the vehicle controls survived on test until the termination of the study.

There were adequate numbers of female rats at risk from late-developing tumors, as 72 percent (36/50) of the high dose, 74 percent (37/50) of the low dose, and 80 percent (16/20) of the vehicle controls survived on test until the termination of the study.

C. Pathology

Histopathologic findings on neoplasms in rats are summarized in Appendix A (Tables A1 and A2); findings on nonneoplastic lesions are summarized in Appendix C (Tables C1 and C2).



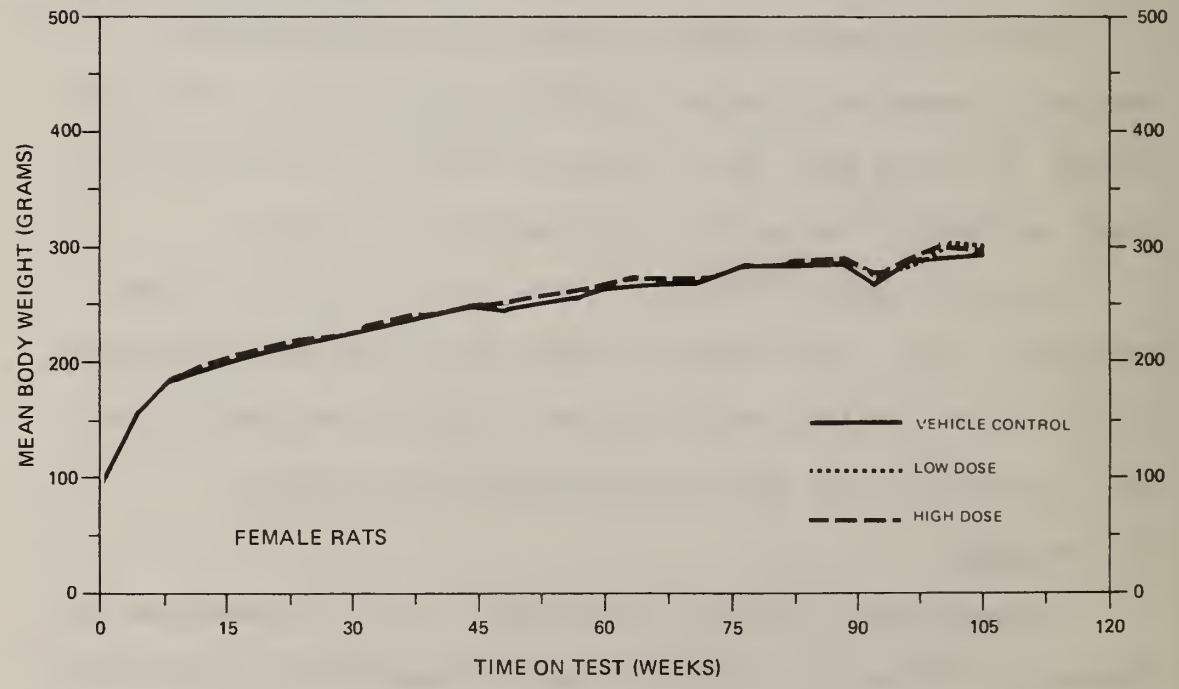


FIGURE 2
GROWTH CURVES FOR 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE CHRONIC STUDY RATS

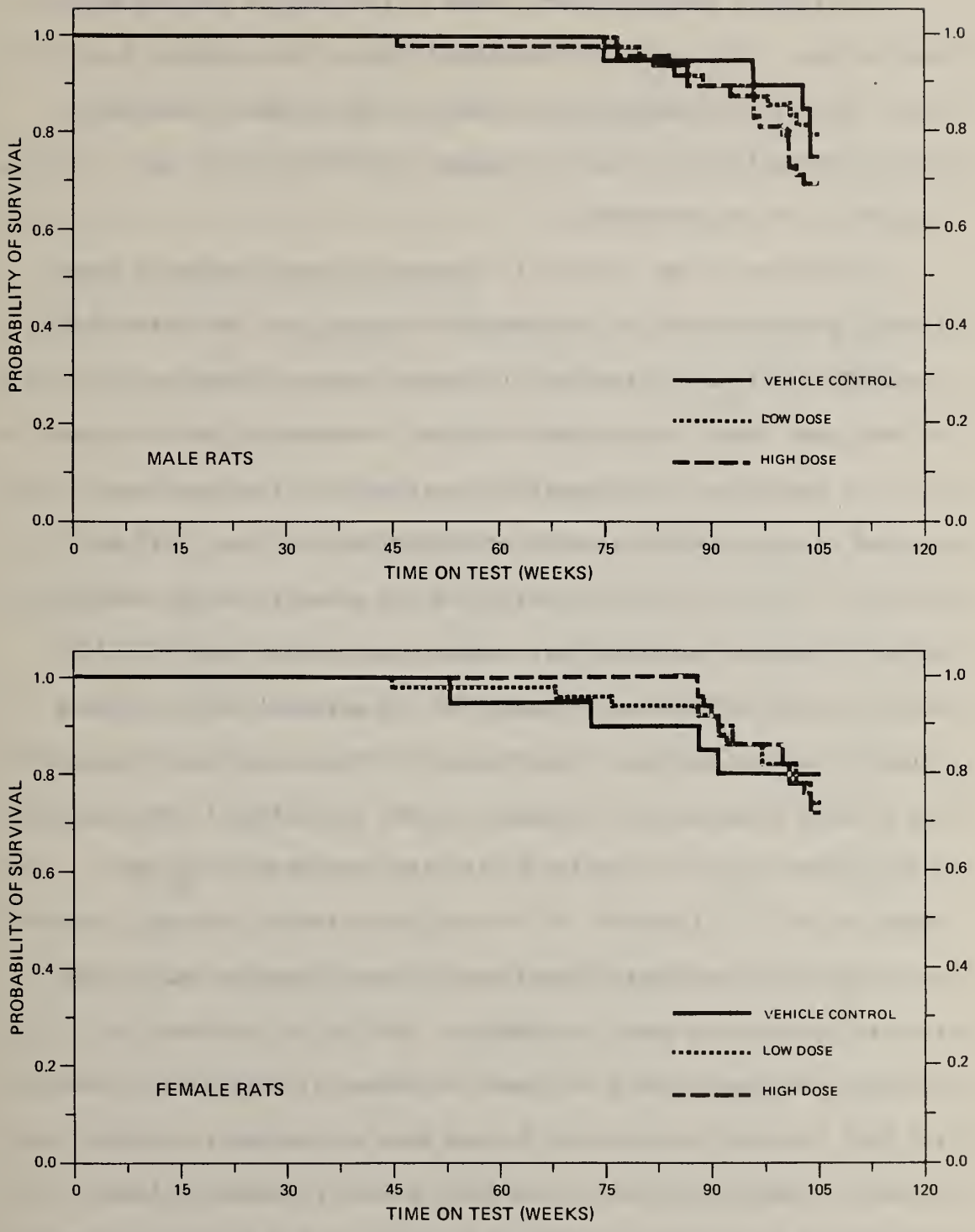


FIGURE 3
SURVIVAL COMPARISONS OF 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE CHRONIC STUDY RATS

A variety of tumors occurred both in the vehicle control and dosed groups. Some types of neoplasms occurred with greater frequency in rats of dosed groups as compared with vehicle controls. However, these lesions are not uncommon in this strain of rat independent of any treatment.

In addition to the neoplastic lesions, a large number of degenerative, proliferative and inflammatory changes were encountered also in animals of the dosed and vehicle control groups (Appendix C). For the most part these nonneoplastic lesions are commonly seen in aged rats. An exception is the gastric hyperplasia of the forestomach observed in both vehicle control and dosed groups (i.e., 5/20 [25 percent], 27/49 [55 percent], and 22/49 [45 percent] in the vehicle control, low dose, and high dose males, respectively, and 3/20 [15 percent], 19/50 [38 percent], and 15/50 [30 percent] in the vehicle control, low dose and high dose females). This lesion was characterized by mild squamous-cell hyperplasia most frequently in the region of the gastric ridge. Associated with the hyperplastic mucosal change was mild inflammation of the subjacent lamina propria. This lesion has been encountered previously in other studies and is probably related to the gavage technique. That it is difficult to interpret the significance of these incidences is suggested by the fact that the occurrence of the lesion does not appear to be dose-dependent. More importantly, the focal nature of these lesions, coupled with the random sampling of the stomach and lack of squamous

stomach in gastric sections from some animals suggests these differences should be viewed with caution.

Based on the results of this pathology examination, it was concluded that 2-(chloromethyl)pyridine HCl was not carcinogenic in Fischer 344 rats under the conditions of this bioassay.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the vehicle control or 2-(chloromethyl)pyridine HCl-dosed groups and where such tumors were observed in at least 5 percent of the group.

For male rats the Cochran-Armitage test indicated a significant ($P = 0.019$) positive association between dose and the incidence of fibromas of the subcutaneous tissue. However, neither of the Fisher exact tests was significant. None of the statistical tests indicated a significant positive association between dose and tumor incidence at any site in female rats.

The Cochran-Armitage test did indicate a significant negative association between dose and the combined incidence of hepatocellular carcinomas or neoplastic nodules of the liver. The departure from linear trend was also significant due to the high incidence in the vehicle control as compared to the zero incidence in the dosed groups. Both the Fisher exact tests comparing high dose to vehicle

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE[a]

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma[b]	0/20(0.00)	0/50(0.00)	5/49(0.10)
P Values[c]	P = 0.019	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.536
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	77
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	4/20(0.20)	12/50(0.24)	11/49(0.22)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.200	1.122
Lower Limit	---	0.429	0.392
Upper Limit	---	4.650	4.404
Weeks to First Observed Tumor	103	80	84
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	3/20(0.15)	0/50(0.00)	0/49(0.00)
P Values[c]	P = 0.005(N)	P = 0.021(N)	P = 0.022(N)
Departure from Linear Trend[e]	P = 0.013	---	---
Relative Risk (Control)[d]	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	0.659	0.673
Weeks to First Observed Tumor	75	---	---

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma or Acidophil Adenoma[b]	3/20(0.15)	6/43(0.14)	5/42(0.12)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.930	0.794
Lower Limit	---	0.228	0.176
Upper Limit	---	5.330	4.742
Weeks to First Observed Tumor	105	77	101
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant[b]	4/20(0.20)	8/50(0.16)	8/48(0.17)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.800	0.833
Lower Limit	---	0.250	0.261
Upper Limit	---	3.327	3.459
Weeks to First Observed Tumor	96	87	96
Thyroid: C-Cell Adenoma[b]	1/19(0.05)	5/49(0.10)	2/48(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.939	0.792
Lower Limit	---	0.243	0.045
Upper Limit	---	89.722	45.751
Weeks to First Observed Tumor	105	105	105

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Pancreatic Islets: Islet-Cell Adenoma[b]	2/20(0.10)	4/50(0.08)	3/48(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.800	0.625
Lower Limit	---	0.128	0.079
Upper Limit	---	8.436	7.137
Weeks to First Observed Tumor	103	80	105
Testis: Interstitial-Cell Tumor[b]	19/20(0.95)	44/49(0.90)	43/49(0.88)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.945	0.924
Lower Limit	---	0.879	0.859
Upper Limit	---	1.169	1.160
Weeks to First Observed Tumor	75	80	85

[a]Treated groups received doses of 75 or 150 mg/kg by gavage 3 days per week.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE[a]

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	2/20(0.10)	6/50(0.12)	6/50(0.12)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.200	1.200
Lower Limit	---	0.243	0.243
Upper Limit	---	11.574	11.574
Weeks to First Observed Tumor	73	97	90
Pituitary: Chromophobe Adenoma[b]	7/19(0.37)	19/48(0.40)	22/44(0.50)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.074	1.357
Lower Limit	---	0.542	0.706
Upper Limit	---	2.594	3.157
Weeks to First Observed Tumor	88	76	88
Thyroid: C-Cell Carcinoma[b]	0/20(0.00)	3/49(0.06)	1/49(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.255	0.023
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	102	105

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Pancreatic Islets: Islet-Cell Adenoma[b]	0/20(0.00)	3/48(0.06)	1/49(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.261	0.023
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	105	88
Mammary Gland: Fibroadenoma[b]	1/20(0.05)	4/50(0.08)	9/50(0.18)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.600	3.600
Lower Limit	---	0.175	0.561
Upper Limit	---	77.169	154.106
Weeks to First Observed Tumor	88	68	88
Uterus: Endometrial Stromal Polyp[b]	3/20(0.15)	6/50(0.12)	13/50(0.26)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.800	1.733
Lower Limit	---	0.195	0.556
Upper Limit	---	4.615	8.773
Weeks to First Observed Tumor	105	104	100

TABLE 4 (CONCLUDED)

[a]Treated groups received doses of 75 or 150 mg/kg by gavage 3 days per week.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

control and low dose to vehicle control also indicated a significant negative association.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 3 and 4, the value one is included; this indicates the absence of statistically significant results. Is should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in rats by 2-(chloromethyl)pyridine HCl that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

High dose male mice had mean body weight depression relative to the vehicle controls while female mice evidenced dose-related mean body weight depression (Figure 4).

No other clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female mice in the vehicle control and 2-(chloromethyl)pyridine HCl-dosed groups are shown in Figure 5. The Tarone test for association between dosage and mortality was not significant for either male or female mice.

There were adequate numbers of male mice at risk from late-developing tumors, as 58 percent (29/50) of the high dose, 72 percent (36/50) of the low dose and 65 percent (13/20) of the vehicle controls survived on test until termination of the study.

There were adequate numbers of female mice at risk from late-developing tumors, as 66 percent (33/50) of the high dose, 80 percent (40/50) of the low dose and 80 percent (16/20) of the vehicle controls survived on test until the termination of the study. One low dose female was missing in week 8.

C. Pathology

Histopathologic findings on neoplasms in mice are summarized in Appendix B (Tables B1 and B2); findings on nonneoplastic lesions are summarized in Appendix D (Tables D1 and D2).

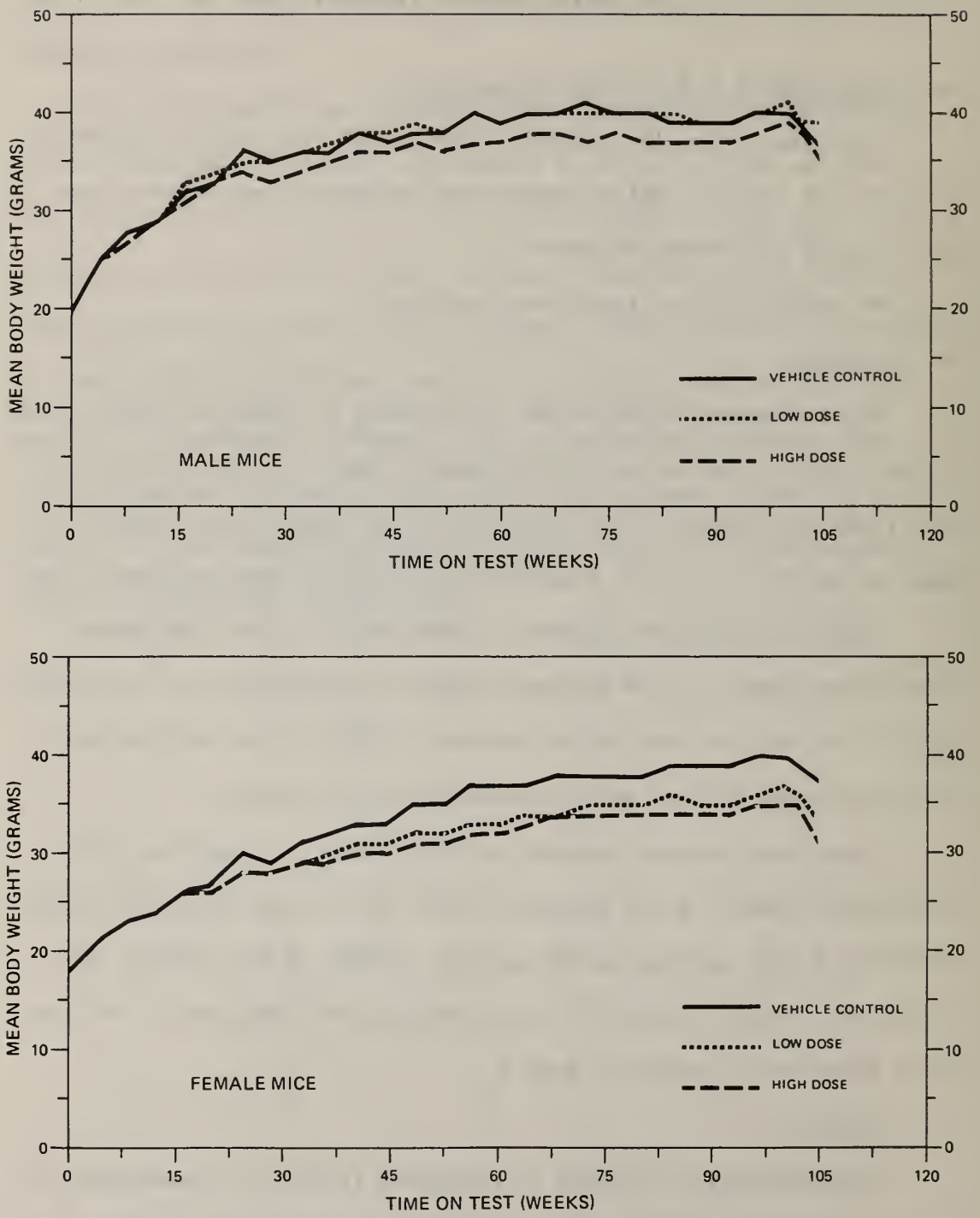
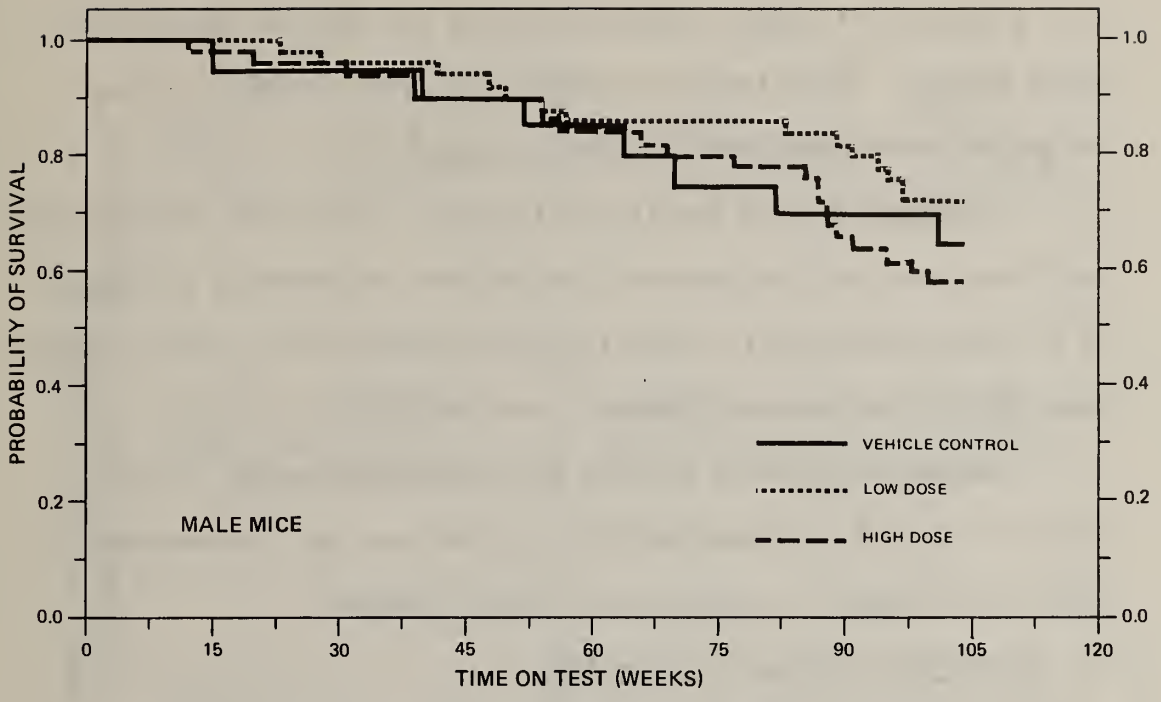


FIGURE 4
GROWTH CURVES FOR 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE CHRONIC STUDY MICE



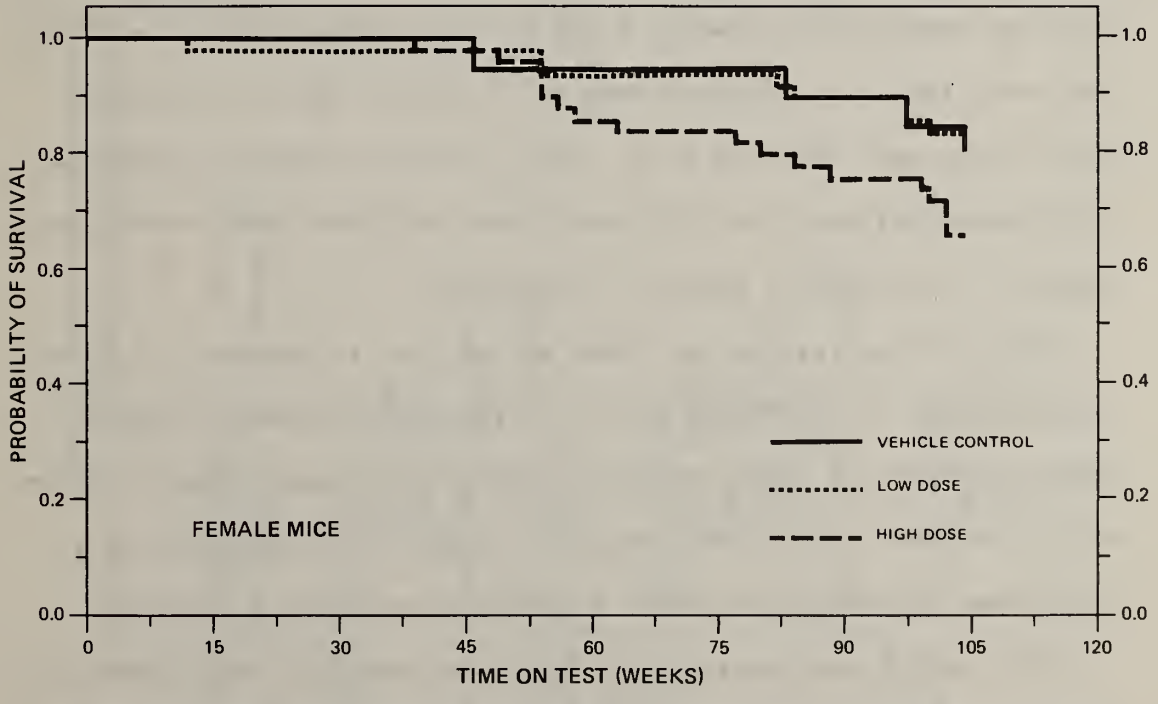


FIGURE 5
SURVIVAL COMPARISONS OF 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE CHRONIC STUDY MICE

A variety of tumors occurred both in the vehicle control and dosed groups. These lesions, however, are not uncommon in this strain of mouse independent of any treatment.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory lesions were encountered in animals of the dosed and vehicle control groups (Appendix D). Most of these nonneoplatic lesions are commonly seen in mice.

Based on the results of this pathology examination, it was concluded that 2-(chloromethyl)pyridine HCl was not carcinogenic in B6C3F1 mice under the conditions of this bioassay.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the vehicle control or 2-(chloromethyl)pyridine HCl-dosed groups and where such tumors were observed in at least 5 percent of the group.

None of the statistical tests at any site in the mice of either sex indicated a significant positive association between chemical administration and tumor incidence. Based upon these results, there was no evidence that 2-(chloromethyl)pyridine hydrochloride was a carcinogen in B6C3F1 mice under the conditions of this bioassay.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative

TABLE 5

TIME-ADJUSTED ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE[a,e]

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	2/17(0.12)	5/44(0.11)	5/43(0.12)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.966	0.988
Lower Limit	---	0.182	0.186
Upper Limit	---	9.590	9.804
Weeks to First Observed Tumor	104	83	104
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	4/18(0.22)	7/45(0.16)	4/45(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.700	0.400
Lower Limit	---	0.211	0.086
Upper Limit	---	2.963	1.965
Weeks to First Observed Tumor	82	57	89
Liver: Hepatocellular Carcinoma[b]	0/17(0.00)	5/43(0.12)	2/43(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.526	0.123
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	89	104

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	3/17(0.18)	6/43(0.14)	4/43(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.791	0.527
Lower Limit	---	0.198	0.103
Upper Limit	---	4.504	3.330
Weeks to First Observed Tumor	70	89	104

[a]Treated groups received doses of 125 or 250 mg/kg by gavage 3 days per week.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]These analyses were based solely upon animals surviving at least 52 weeks.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE[a]

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	1/19(0.05)	1/49(0.02)	3/48(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.388	1.187
Lower Limit	---	0.005	0.105
Upper Limit	---	29.845	61.031
Weeks to First Observed Tumor	104	104	102
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	3/20(0.15)	8/49(0.16)	4/50(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.088	0.533
Lower Limit	---	0.301	0.102
Upper Limit	---	5.926	3.410
Weeks to First Observed Tumor	97	82	100

[a]Treated groups received doses of 125 or 250 mg/kg by gavage 3 days per week.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 5 and 6, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in mice by 2-(chloromethyl)pyridine HCl that could not be established under the conditions of this test.

V. DISCUSSION

There were no significant positive associations between the dosages of 2-(chloromethyl)pyridine hydrochloride administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Mean body weight depression was observed in dosed mice of both sexes when compared to the vehicle controls, indicating that the dosages of 2-(chloromethyl)pyridine hydrochloride administered to these animals in this bioassay may have approximated the maximum tolerated concentrations. Since no distinct mean body weight depression relative to controls, no significant accelerated mortality, and no other signs of toxicity were associated with administration of 2-(chloromethyl)pyridine hydrochloride to rats, it is possible that these animals may have been able to tolerate a higher dosage.

None of the statistical tests for any site in female rats or in mice of either sex indicated a significant positive association between compound administration and tumor incidence. There was a significant positive trend between the dosages administered and the incidences of subcutaneous fibromas in male rats. The Fisher exact comparisons, however, were not significant.

Under the conditions of this bioassay, administration of 2-(chloromethyl)pyridine hydrochloride was not carcinogenic to Fischer 344 rats or B6C3F1 mice.

VI. BIBLIOGRAPHY

Armitage, P., Statistical Methods in Medical Research, Chapter 14. J. Wiley & Sons, New York, 1971.

Berenblum, I., editor, Carcinogenicity Testing. International Union Against Cancer, Technical Report Series, Vol. 2. International Union Against Cancer, Geneva, 1969.

Chemical Abstracts Service, The Chemical Abstracts Service (CAS) Ninth Collective Index, Volumes 76-85, 1972-1976. American Chemical Society, Washington, D.C., 1977.

Cox, D.R., Analysis of Binary Data, Chapters 4 and 5. Methuen and Co., Ltd., London, 1970.

Cox, D.R., "Regression Models and Life-Tables." Journal of the Royal Statistical Society, Series "B" 34:187-220, 1972.

Fuhlhage, D.W., "Nematocidal Picolyl Chlorides." U.S. Patent 3,539,688 (Thomas-Hayward Chemical Co.), November 10, 1970; Chemical Abstracts 74, 31076m.

Gart, J.J., "The Comparison of Proportions: A Review of Significance Tests, Confidence Limits, and Adjustments for Stratification." International Statistical Institute Review 39:148-169, 1971.

Haas, G. and A. Sallmann, "Pyridylalkyl (2-anilinophenyl) acetates." Ger. Offen. 2,339,862 (Ciba-Geigy A.-G.), February 28, 1974; Chemical Abstracts 80, 146029g.

Haas, G. and A. Sallman, "Substituted Anilino-phenylacetic-acid-(2,3- or 4-pyridyl)-methyl esters and Derivatives." U.S. Patent 3,897,437 (Ciba-Geigy Corp.), July 29, 1975; Chemical Abstracts 83, 178848j.

Harris, P.N., "Induction of Carcinoma of the Forestomach by 2-(α,β-Dichloroethyl)-pyridine HCl (CLEP)." Proceedings of the American Association for Cancer Research 9:28, 1968.

Kaplan, E.L., and P. Meier, "Nonparametric Estimation from Incomplete Observations." Journal of the American Statistical Association 53:457-481, 1958.

Linhart, M.S., J.A. Cooper, R.L. Martin, N.P. Page, and J.A. Peters, "Carcinogenesis Bioassay Data System." Computers and Biomedical Research 7:230-248, 1974.

Matsumura, E., T. Nashima, and F. Ishibashi, "Synthesis of 1-Methyl-2-pyridones from 2-Chloromethylpyridines." Bulletin of the Chemical Society of Japan 43(11):3540-3542, 1970

Miller, R.G., Simultaneous Statistical Inference. McGraw-Hill Book Co., New York, 1966.

Nakanishi, M., T. Munakata, H. Maruyama, and N. Setoguchi, "Piperazine Derivatives." Japan. 73 43,512 (Yoshitomi Pharmaceutical Industries, Ltd.), December 19, 1973; Chemical Abstracts 79, 133502p.

Saffiotti, U., R. Montesano, A.R. Sellakumar, F. Cefis, and D.G. Kaufman, "Respiratory Tract Carcinogenesis in Hamsters Induced by Different Numbers of Administration of Benzo (a) Pyrene and Ferric Oxide." Cancer Research 32:1073-1079, 1972.

Stevens, R., J.A. Baker, and J.W. James, "Barbituric Acid Derivatives." Ger. Offen. 2,205,253 (Aspro-Nicholas, Ltd.), August 16, 1973a; Chemical Abstracts 79, 115612z.

Stevens, R., J.A. Baker, and J.W. James, "5,5-Disubstituted Barbituric Acids." British Patent 1,310,234 (Aspro-Nicholas, Ltd.) March 14, 1973b; Chemical Abstracts 78, 147991t.

Tarone, R.E., "Tests for Trend in Life-Table Analysis." Biometrika 62:679-682, 1975.

Tilles, H. and M.E. Brokke, "Herbicidal S-(Pyridylmethyl) Thiocarbamates." U.S. Patent 3,704,236 (Stauffer Chemical Co.), November 28, 1972; Chemical Abstracts 78, 43280u.

U.S. International Trade Commission, Synthetic Organic Chemicals: United States Production and Sales, 1976. USITC Publication 833, U.S. Government Printing Office, Washington, D.C., 1977.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE

	CONTROL (VEH) 11-1445	LOW DOSE 11-1443	HIGH DOSE 11-1441
ANIMALS INITIALLY IN STUDY	20	50	@50
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(49)
PAPILLOMA, NOS	1 (5%)		
BASAL-CELL TUMOR		2 (4%)	
TRICHOEPITHELIOMA		1 (2%)	1 (2%)
SEBACEOUS ADENOMA			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(49)
FIBROMA			5 (10%)
FIBROSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(48)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	1 (2%)
SARCOMA, NOS, METASTATIC	1 (5%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(49)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
LEUKEMIA,NOS		5 (10%)	5 (10%)
UNDIFFERENTIATED LEUKEMIA	3 (15%)	2 (4%)	3 (6%)
GRANULOCYTIC LEUKEMIA		5 (10%)	
MONOCYTIC LEUKEMIA			1 (2%)
#SPLEEN	(20)	(50)	(49)
LEIOMYOMA			1 (2%)
#MEDIASTINAL L.NODE	(20)	(50)	(48)
SARCOMA, NOS, METASTATIC	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS
50 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS FOUND TO BE A FEMALE IN A MALE

TABLE A1 (CONTINUED)

	CONTROL (VEH) 11-1445	LOW DOSE 11-1443	HIGH DOSE 11-1441
#LUNG	(20)	(49)	(48)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
CIRCULATORY SYSTEM			
*BLOOD VESSEL	(20)	(50)	(49)
PHEOCHROMOCYTOMA, METASTATIC		1 (2%)	
DIGESTIVE SYSTEM			
*ORAL CAVITY	(20)	(50)	(49)
MYXOSARCOMA		1 (2%)	
#LIVER	(20)	(50)	(49)
NEOPLASTIC NODULE	2 (10%)		
HEPATOCELLULAR CARCINOMA	1 (5%)		
#PANCREAS	(20)	(50)	(48)
ACINAR-CELL ADENOMA		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(43)	(42)
CHROMOPHOBE ADENOMA	3 (15%)	6 (14%)	4 (10%)
ACIDOPHIL ADENOMA			1 (2%)
#ADRENAL	(20)	(50)	(48)
CORTICAL ADENOMA			1 (2%)
PHEOCHROMOCYTOMA	2 (10%)	6 (12%)	7 (15%)
PHEOCHROMOCYTOMA, MALIGNANT	2 (10%)	2 (4%)	1 (2%)
#THYROID	(19)	(49)	(48)
FOLLICULAR-CELL ADENOMA			1 (2%)
C-CELL ADENOMA	1 (5%)	5 (10%)	2 (4%)
#PANCREATIC ISLETS	(20)	(50)	(48)
ISLET-CELL ADENOMA	2 (10%)	4 (8%)	3 (6%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONTINUED)

	CONTROL (VEH) 11-1445	LOW DOSE 11-1443	HIGH DOSE 11-1441
REPRODUCTIVE SYSTEM			
#TESTIS	(20)	(49)	(49)
INTERSTITIAL-CELL TUMOR	19 (95%)	44 (90%)	43 (88%)
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(49)
GLIOMA, NOS			1 (2%)
OLIGODENDROGLIOMA	1 (5%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(20)	(50)	(49)
SARCOMA, NOS, INVASIVE	1 (5%)		
BODY CAVITIES			
*MEDIASTINUM	(20)	(50)	(49)
MESOTHELIOMA, NOS	1 (5%)		
*PERITONEUM	(20)	(50)	(49)
MESOTHELIOMA, NOS			1 (2%)
ALL OTHER SYSTEMS			
SITE UNKNOWN			
SARCOMA, NOS	1		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONCLUDED)

	CONTROL (VEH) 11-1445	LOW DOSE 11-1443	HIGH DOSE 11-1441
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	5	6
MORIBUND SACRIFICE	4	5	10
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	15	40	33
ANIMAL MISSING			
ANIMAL DELETED(WRONG SEX)			1
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	49	48
TOTAL PRIMARY TUMORS	40	85	86
TOTAL ANIMALS WITH BENIGN TUMORS	20	49	45
TOTAL BENIGN TUMORS	28	69	70
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	16	14
TOTAL MALIGNANT TUMORS	9	16	15
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	
TOTAL SECONDARY TUMORS	3	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	2		1
TOTAL UNCERTAIN TUMORS	3		1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE

	CONTROL(VEH) 11-1446	LOW DOSE 11-1444	HIGH DOSE 11-1442
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
KERATOACANTHOMA		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
FIBROMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(49)
CARCINOMA, NOS, METASTATIC			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
C-CELL CARCINOMA, METASTATIC		2 (4%)	
OSTEOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
LEUKEMIA,NOS	1 (5%)	4 (8%)	3 (6%)
UNDIFFERENTIATED LEUKEMIA		1 (2%)	1 (2%)
GRANULOCYTIC LEUKEMIA			2 (4%)
MONOCYTIC LEUKEMIA	1 (5%)		
#SPLEEN	(20)	(49)	(49)
UNDIFFERENTIATED LEUKEMIA		1 (2%)	
#MANDIBULAR L. NODE	(20)	(50)	(50)
C-CELL CARCINOMA, METASTATIC		1 (2%)	
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A2 (CONTINUED)

	CONTROL(VEH) 11-1446	LOW DOSE 11-1444	HIGH DOSE 11-1442
DIGESTIVE SYSTEM			
#STOMACH	(20)	(50)	(50)
FIBROMA		1 (2%)	
#SMALL INTESTINE	(20)	(49)	(50)
LEIOMYOSARCOMA		2 (4%)	
URINARY SYSTEM			
#URINARY BLADDER	(18)	(47)	(45)
OSTEOSARCOMA, INVASIVE		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(48)	(44)
CHROMOPHOBE ADENOMA	7 (37%)	19 (40%)	22 (50%)
#ADRENAL	(20)	(49)	(49)
CORTICAL ADENOMA	2 (10%)		
CORTICAL CARCINOMA		1 (2%)	
PHEOCHROMOCYTOMA			1 (2%)
#THYROID	(20)	(49)	(49)
C-CELL CARCINOMA		3 (6%)	1 (2%)
CYSTADENOMA, NOS		1 (2%)	
#PANCREATIC ISLETS	(20)	(48)	(49)
ISLET-CELL ADENOMA		3 (6%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOMA, NOS		1 (2%)	1 (2%)
PAPILLARY CYSTADENOMA, NOS		1 (2%)	
FIBROADENOMA	1 (5%)	4 (8%)	9 (18%)
#UTERUS	(20)	(50)	(50)
FIBROMA			1 (2%)
LEIOMYOSARCOMA			1 (2%)
ENDOMETRIAL STROMAL POLYP	3 (15%)	6 (12%)	13 (26%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 (CONTINUED)

	CONTROL(VEH) 11-1446	LOW DOSE 11-1444	HIGH DOSE 11-1442
#OVARY	(20)	(50)	(50)
C-CELL CARCINOMA, METASTATIC		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(50)
ASTROCYTOMA		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*FEMUR	(20)	(50)	(50)
OSTEOSARCOMA		1 (2%)	
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	4	5
MORIBUND SACRIFICE	2	9	9
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	37	36
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 (CONCLUDED)

	CONTROL (VEH) 11-1446	LOW DOSE 11-1444	HIGH DOSE 11-1442
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	35	38
TOTAL PRIMARY TUMORS	15	52	58
TOTAL ANIMALS WITH BENIGN TUMORS	10	28	34
TOTAL BENIGN TUMORS	13	38	49
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	12	7
TOTAL MALIGNANT TUMORS	2	14	9
TOTAL ANIMALS WITH SECONDARY TUMORS#		3	1
TOTAL SECONDARY TUMORS		6	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE

	CONTROL (VEH) 22-2445	LOW DOSE 22-2443	HIGH DOSE 22-2441
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SARCOMA, NOS	1 (5%)	1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(19)	(49)	(48)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (11%)	5 (10%)	5 (10%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	1 (2%)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (5%)	1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		2 (4%)	1 (2%)
LEUKEMIA,NOS	1 (5%)		
#MESENTERIC L. NODE	(14)	(42)	(37)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (7%)		
MALIGNANT LYMPHOMA, MIXED TYPE			2 (5%)
#LUNG	(19)	(49)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)		
#LIVER	(19)	(48)	(48)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#ILEUM	(19)	(47)	(48)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL (VEH) 22-2445	LOW DOSE 22-2443	HIGH DOSE 22-2441
DIGESTIVE SYSTEM			
#LIVER	(19)	(48)	(48)
HEPATOCELLULAR ADENOMA	3 (16%)	1 (2%)	2 (4%)
HEPATOCELLULAR CARCINOMA		5 (10%)	2 (4%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(20)	(50)	(50)
PAPILLARY ADENOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
SARCOMA, NOS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1 (CONCLUDED)

	CONTROL (VEH) 22-2445	LOW DOSE 22-2443	HIGH DOSE 22-2441
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	6	11	21
MORIBUND SACRIFICE	1	3	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	13	36	29
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	9	18	13
TOTAL PRIMARY TUMORS	10	20	15
TOTAL ANIMALS WITH BENIGN TUMORS	5	6	8
TOTAL BENIGN TUMORS	5	6	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	13	7
TOTAL MALIGNANT TUMORS	5	14	7
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	
TOTAL SECONDARY TUMORS		2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE

	CONTROL(VEH) 22-2446	LOW DOSE 22-2444	HIGH DOSE 22-2442
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(49)	(50)
SARCOMA, NOS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(19)	(49)	(48)
HEPATOCELLULAR CARCINOMA, METAST	1 (5%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	1 (2%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)	3 (6%)	1 (2%)
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (10%)	1 (2%)	
LEUKEMIA,NOS		1 (2%)	
#MESENTERIC L. NODE	(18)	(46)	(37)
MALIG.LYMPHOMA, UNDIFFER-TYPE			2 (5%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#SMALL INTESTINE	(20)	(48)	(47)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B2 (CONTINUED)

	CONTROL (VEH) 22-2446	LOW DOSE 22-2444	HIGH DOSE 22-2442
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(49)
HEPATOCELLULAR ADENOMA		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(49)
HEMANGIOMA			1 (2%)
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(49)	(46)
ADENOCARCINOMA, NOS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(49)	(50)
OSTEOSARCOMA			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2 (CONCLUDED)

	CONTROL (VEH) 22-2446	LOW DOSE 22-2444	HIGH DOSE 22-2442
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	8	16
MORIBUND SACRIFICE		1	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	40	33
ANIMAL MISSING		1	
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	11	10
TOTAL PRIMARY TUMORS	4	11	10
TOTAL ANIMALS WITH BENIGN TUMORS	1	2	3
TOTAL BENIGN TUMORS	1	2	3
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	9	7
TOTAL MALIGNANT TUMORS	3	9	7
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		
TOTAL SECONDARY TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE

	CONTROL (VEH) 11-1445	LOW DOSE 11-1443	HIGH DOSE 11-1441
ANIMALS INITIALLY IN STUDY	20	50	@50
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(49)
EPIDERMAL INCLUSION CYST			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(48)
CONGESTION, NOS		3 (6%)	1 (2%)
CONGESTION, CHRONIC PASSIVE		1 (2%)	
EDEMA, NOS			1 (2%)
PNEUMONIA, CHRONIC MURINE		13 (27%)	12 (25%)
NODULE			1 (2%)
HYPERPLASIA, FOCAL			1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM			3 (6%)
MONOCYTOSIS	1 (5%)		
#LUNG/ALVEOLI	(20)	(49)	(48)
HYPERTROPHY, NOS			1 (2%)
HYPERTROPHY, FOCAL			3 (6%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(49)	(47)
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	
#SPLEEN	(20)	(50)	(49)
CONGESTION, NOS	1 (5%)		
SCLEROSIS		1 (2%)	
HEMATOPOIESIS	2 (10%)		
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(48)
THROMBUS, ORGANIZED		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS
50 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS FOUND TO BE A FEMALE IN A MALE

TABLE C1 (CONTINUED)

	CONTROL (VEH) 11-1445	LOW DOSE 11-1443	HIGH DOSE 11-1441
#HEART/ATRIUM	(20)	(50)	(48)
THROMBOSIS, NOS		1 (2%)	1 (2%)
THROMBUS, MURAL		1 (2%)	
#MYOCARDIUM	(20)	(50)	(48)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, CHRONIC	1 (5%)		
INFLAMMATION, CHRONIC FOCAL	2 (10%)	1 (2%)	1 (2%)
FIBROSIS	4 (20%)	31 (62%)	24 (50%)
FIBROSIS, DIFFUSE		1 (2%)	
#CARDIAC VALVE	(20)	(50)	(48)
THROMBOSIS, NOS			1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(50)	(47)
NUCLEAR ENLARGEMENT		1 (2%)	
#LIVER	(20)	(50)	(49)
CONGESTION, NOS			1 (2%)
CONGESTION, CHRONIC PASSIVE		1 (2%)	
NECROSIS, FOCAL		1 (2%)	
METAMORPHOSIS FATTY		1 (2%)	3 (6%)
BASOPHILIC CYTO CHANGE	3 (15%)	2 (4%)	
HYPERPLASIA, FOCAL		2 (4%)	1 (2%)
#LIVER/CENTRILOBULAR	(20)	(50)	(49)
METAMORPHOSIS FATTY	1 (5%)		
#LIVER/HEPATOCYTES	(20)	(50)	(49)
HYPERPLASIA, FOCAL		1 (2%)	
#BILE DUCT	(20)	(50)	(49)
FIBROSIS	1 (5%)		
HYPERPLASIA, NOS	8 (40%)	14 (28%)	5 (10%)
#PANCREAS	(20)	(50)	(48)
FIBROSIS, FOCAL			2 (4%)
PERIARTERITIS			2 (4%)
NECROSIS, FAT		1 (2%)	
ATROPHY, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONTINUED)

	CONTROL (VEH) 11-1445	LOW DOSE 11-1443	HIGH DOSE 11-1441
ATROPHY, FOCAL		4 (8%)	
#PANCREATIC ACINUS	(20)	(50)	(48)
ATROPHY, NOS	1 (5%)	2 (4%)	3 (6%)
ATROPHY, FOCAL	2 (10%)		
#STOMACH	(20)	(49)	(49)
INFLAMMATION, CHRONIC		1 (2%)	
CALCIFICATION, FOCAL			1 (2%)
#GASTRIC MUCOSA	(20)	(49)	(49)
HYPERPLASIA, NOS	5 (25%)	27 (55%)	22 (45%)
#COLON	(20)	(49)	(47)
PARASITISM	2 (10%)	10 (20%)	4 (9%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(48)
HAMARTOMA		1 (2%)	
GLOMERULONEPHRITIS, NOS	2 (10%)		
INFLAMMATION, CHRONIC	4 (20%)	42 (84%)	33 (69%)
NEPHROPATHY, TOXIC	6 (30%)	5 (10%)	6 (13%)
#KIDNEY/TUBULE	(20)	(50)	(48)
NECROSIS, FOCAL	2 (10%)		
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(43)	(42)
PERSISTENT EMBRYONIC STRUCTURE		1 (2%)	
CYST, NOS			1 (2%)
#ADRENAL	(20)	(50)	(48)
LIPOIDOSIS		1 (2%)	
#ADRENAL CORTEX	(20)	(50)	(48)
METAMORPHOSIS FATTY		1 (2%)	
HYPERPLASIA, FOCAL		1 (2%)	
#ADRENAL MEDULLA	(20)	(50)	(48)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONTINUED)

	CONTROL(VEH) 11-1445	LOW DOSE 11-1443	HIGH DOSE 11-1441
#THYROID	(19)	(49)	(48)
ABSCESS, NOS			1 (2%)
HYPERPLASIA, C-CELL			4 (8%)
#PANCREATIC ISLETS	(20)	(50)	(48)
HYPERPLASIA, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
#PROSTATE	(19)	(47)	(47)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
#TESTIS	(20)	(49)	(49)
NECROSIS, NOS	1 (5%)		
ATROPHY, NOS	3 (15%)	1 (2%)	1 (2%)
NERVOUS SYSTEM			
#CEREBRUM	(20)	(50)	(49)
DILATATION, NOS			1 (2%)
HEMORRHAGE			2 (4%)
MALACIA			1 (2%)
#BRAIN	(20)	(50)	(49)
CONGESTION, NOS		1 (2%)	
HEMORRHAGE		1 (2%)	
INFARCT, NOS			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MEDIASTINUM	(20)	(50)	(49)
INFLAMMATION, CHRONIC NECROTIZIN	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONCLUDED)

	CONTROL(VEH) 11-1445	LOW DOSE 11-1443	HIGH DOSE 11-1441
*ABDOMINAL CAVITY	(20)	(50)	(49)
NECROSIS, FAT		3 (6%)	1 (2%)
*MESENTERY	(20)	(50)	(49)
STEATITIS		1 (2%)	1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(49)
LEUKEMOID REACTION		3 (6%)	
THORAX			
NECROSIS, FAT			1
SPECIAL MORPHOLOGY SUMMARY			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS TREATED WITH 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE

	CONTROL(VEH) 11-1446	LOW DOSE 11-1444	HIGH DOSE 11-1442
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
INFLAMMATION, CHRONIC			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(49)
ATELECTASIS			1 (2%)
CONGESTION, NOS	1 (5%)	1 (2%)	1 (2%)
EDEMA, NOS		1 (2%)	
PNEUMONIA, CHRONIC MURINE		17 (34%)	3 (6%)
GRANULOMA, FOREIGN BODY	1 (5%)		
PERIVASCULAR CUFFING		2 (4%)	
FOAM-CELL			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(49)	(49)
HEMOSIDEROSIS			1 (2%)
HEMATOPOIESIS	1 (5%)		
#MESENTERIC L. NODE	(20)	(50)	(50)
PIGMENTATION, NOS	1 (5%)		
DEPLETION	1 (5%)		
HYPERPLASIA, RETICULUM CELL		1 (2%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(50)	(50)
INFLAMMATION, FOCAL		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C2 (CONTINUED)

	CONTROL(VEH) 11-1446	LOW DOSE 11-1444	HIGH DOSE 11-1442
INFLAMMATION, CHRONIC FOCAL	2 (10%)	1 (2%)	4 (8%)
FIBROSIS	4 (20%)	13 (26%)	9 (18%)
#ENDOCARDIUM	(20)	(50)	(50)
INFLAMMATION, FOCAL	1 (5%)		
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, ACUTE	1 (5%)		
ATROPHY, DIFFUSE		1 (2%)	
METAPLASIA, SQUAMOUS		1 (2%)	
#LIVER	(20)	(50)	(50)
DEGENERATION, NOS		2 (4%)	
METAMORPHOSIS FATTY	1 (5%)	3 (6%)	1 (2%)
BASOPHILIC CYTO CHANGE	1 (5%)	14 (28%)	16 (32%)
FOCAL CELLULAR CHANGE	5 (25%)	15 (30%)	1 (2%)
HYPERPLASIA, FOCAL		3 (6%)	
LEUKOCYTOSIS, NEUTROPHILIC			1 (2%)
HYPERPLASIA, RETICULUM CELL		1 (2%)	
#LIVER/HEPATOCYTES	(20)	(50)	(50)
FOCAL CELLULAR CHANGE	1 (5%)		
HYPERPLASIA, FOCAL			1 (2%)
*BILE DUCT	(20)	(50)	(50)
HYPERPLASIA, NOS	3 (15%)	3 (6%)	6 (12%)
#PANCREAS	(20)	(48)	(49)
FIBROSIS		1 (2%)	
NECROSIS, FAT			1 (2%)
ATROPHY, FOCAL		2 (4%)	
#PANCREATIC ACINUS	(20)	(48)	(49)
ATROPHY, NOS			2 (4%)
ATROPHY, FOCAL	1 (5%)	1 (2%)	1 (2%)
#GASTRIC MUCOSA	(20)	(50)	(50)
HYPERPLASIA, NOS	3 (15%)	19 (38%)	15 (30%)
#LARGE INTESTINE	(20)	(49)	(50)
PARASITISM	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONTINUED)

	CONTROL (VEH) 11-1446	LOW DOSE 11-1444	HIGH DOSE 11-1442
#COLON	(20)	(49)	(50)
PARASITISM	6 (30%)	9 (18%)	5 (10%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
MINERALIZATION		2 (4%)	5 (10%)
INFLAMMATION, CHRONIC	5 (25%)	16 (32%)	14 (28%)
NEPHROPATHY, TOXIC	4 (20%)	4 (8%)	4 (8%)
#KIDNEY/TUBULE	(20)	(50)	(50)
PIGMENTATION, NOS		1 (2%)	
#URINARY BLADDER	(18)	(47)	(45)
CALCULUS, NOS	1 (6%)		
HYPERPLASIA, EPITHELIAL	1 (6%)	1 (2%)	
METAPLASIA, SQUAMOUS	1 (6%)		
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(48)	(44)
CYST, NOS	4 (21%)	2 (4%)	2 (5%)
HEMORRHAGE			1 (2%)
HEMORRHAGIC CYST	1 (5%)		1 (2%)
HYPERPLASIA, CHROMOPHOBE-CELL		1 (2%)	1 (2%)
#ADRENAL	(20)	(49)	(49)
LIPOIDOSIS		1 (2%)	
CYTOPLASMIC VACUOLIZATION	1 (5%)		
#ADRENAL CORTEX	(20)	(49)	(49)
CYST, NOS	1 (5%)		
HYPERPLASIA, NOS		1 (2%)	
#THYROID	(20)	(49)	(49)
HYPERPLASIA, C-CELL		2 (4%)	1 (2%)
#PANCREATIC ISLETS	(20)	(48)	(49)
HYPERPLASIA, FOCAL		1 (2%)	
REPRODUCTIVE SYSTEM			
#MAMMARY GLAND	(20)	(50)	(50)
CYSTIC DUCTS			2 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONCLUDED)

	CONTROL (VEH) 11-1446	LOW DOSE 11-1444	HIGH DOSE 11-1442
HYPERPLASIA, CYSTIC			1 (2%)
#UTERUS	(20)	(50)	(50)
HYDROMETRA	1 (5%)		
CYST, NOS		1 (2%)	
INFLAMMATION, ACUTE		1 (2%)	
#UTERUS/ENDOMETRIUM	(20)	(50)	(50)
HYPERPLASIA, NOS		3 (6%)	
HYPERPLASIA, CYSTIC	1 (5%)		1 (2%)
#OVARY	(20)	(50)	(50)
CYST, NOS		2 (4%)	
FOLLICULAR CYST, NOS		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(50)
HEMORRHAGE	1 (5%)	1 (2%)	
MALACIA		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, GRANULOMATOUS			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	2

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE

	CONTROL(VEH) 22-2445	LOW DOSE 22-2443	HIGH DOSE 22-2441
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
#SKIN	(20)	(50)	(50)
ULCER, NOS	1 (5%)		
DEGENERATION, NOS			1 (2%)
#SUBCUT TISSUE	(20)	(50)	(50)
ABSCESS, NOS	1 (5%)		
GRANULATION, TISSUE		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(19)	(49)	(48)
CONGESTION, NOS	1 (5%)	1 (2%)	8 (17%)
EDEMA, NOS		1 (2%)	6 (13%)
HEMORRHAGE	1 (5%)		
INFLAMMATION, ACUTE		1 (2%)	
PNEUMONIA, CHRONIC MURINE	2 (11%)	6 (12%)	3 (6%)
ALVEOLAR MACROPHAGES			1 (2%)
HISTIOCYTOSIS	1 (5%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(18)	(45)	(46)
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	
#SPLEEN	(19)	(46)	(47)
HYPERPLASIA, LYMPHOID			2 (4%)
#MESENTERIC L. NODE	(14)	(42)	(37)
HYPERPLASIA, RETICULUM CELL		1 (2%)	1 (3%)
CIRCULATORY SYSTEM			
#HEART	(19)	(49)	(48)
ENDOCARDITIS, BACTERIAL	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D1 (CONTINUED)

	CONTROL (VEH) 22-2445	LOW DOSE 22-2443	HIGH DOSE 22-2441
INFLAMMATION, ACUTE		1 (2%)	
*PROSTATIC ARTERY	(20)	(50)	(50)
DEGENERATION, HYALINE		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(18)	(45)	(43)
FIBROSIS		1 (2%)	
ATROPHY, NOS		1 (2%)	
#LIVER	(19)	(48)	(48)
CONGESTION, NOS			1 (2%)
INFLAMMATION, FOCAL		1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
NECROSIS, NOS			2 (4%)
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY		2 (4%)	
CYTOPLASMIC VACUOLIZATION	1 (5%)		
HEMATOPOIESIS			1 (2%)
#LIVER/PERIPORTAL	(19)	(48)	(48)
METAMORPHOSIS FATTY		1 (2%)	
#PANCREAS	(18)	(46)	(47)
DILATATION/DUCTS		1 (2%)	2 (4%)
#PANCREATIC ACINUS	(18)	(46)	(47)
ATROPHY, NOS		2 (4%)	1 (2%)
#SMALL INTESTINE	(19)	(47)	(48)
AMYLOIDOSIS	1 (5%)		1 (2%)
#ILEUM	(19)	(47)	(48)
ULCER, NOS		1 (2%)	
#COLON	(18)	(47)	(47)
PARASITISM	6 (33%)	14 (30%)	12 (26%)
URINARY SYSTEM			
#KIDNEY	(19)	(47)	(48)
HYDRONEPHROSIS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1 (CONTINUED)

	CONTROL (VEH) 22-2445	LOW DOSE 22-2443	HIGH DOSE 22-2441
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)		
INFLAMMATION, SUPPURATIVE		1 (2%)	
PERIARTERITIS			1 (2%)
DEGENERATION, HYALINE			1 (2%)
INFARCT, FOCAL		1 (2%)	
AMYLOIDOSIS	1 (5%)		
#URINARY BLADDER	(17)	(45)	(44)
CALCULUS, NOS			1 (2%)
DISTENTION		1 (2%)	
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#PROSTATE	(19)	(43)	(43)
INFLAMMATION, ACUTE		1 (2%)	
NERVOUS SYSTEM			
*EPENDYMAL CELL	(20)	(50)	(50)
INFLAMMATION, FOCAL		1 (2%)	
#BRAIN	(20)	(49)	(48)
PERIVASCULAR CUFFING		1 (2%)	
CORPORA AMYLACEA	6 (30%)	17 (35%)	11 (23%)
PSAMMOMA BODIES	2 (10%)	4 (8%)	1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(20)	(50)	(50)
INFLAMMATION, ACUTE SUPPURATIVE		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1 (CONCLUDED)

	CONTROL(VEH) 22-2445	LOW DOSE 22-2443	HIGH DOSE 22-2441
*MESENTERY	(20)	(50)	(50)
STEATITIS			2 (4%)
NECROSIS, FAT			3 (6%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
AMYLOIDOSIS			2 (4%)
ADIPOSE TISSUE			
INFLAMMATION, GRANULOMATOUS			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	9	9

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH 2-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE

	CONTROL (VEH) 22-2446	LOW DOSE 22-2444	HIGH DOSE 22-2442
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(19)	(49)	(48)
CONGESTION, NOS		1 (2%)	4 (8%)
EDEMA, NOS			5 (10%)
HEMORRHAGE		1 (2%)	
PNEUMONIA, CHRONIC MURINE	6 (32%)	16 (33%)	11 (23%)
NECROSIS, NOS		1 (2%)	
LEUKOCYTOSIS, NEUTROPHILIC			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(43)	(44)
HYPERPLASIA, NEUTROPHILIC			1 (2%)
#SPLEEN	(18)	(49)	(47)
HYPERPLASIA, RETICULUM CELL		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
#LYMPH NODE	(18)	(46)	(37)
INFLAMMATION, NOS		1 (2%)	
HYPERPLASIA, LYMPHOID			1 (3%)
#MESENTERIC L. NODE	(18)	(46)	(37)
INFLAMMATION, NOS	1 (6%)		
INFLAMMATION, GRANULOMATOUS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL (VEH) 22-2446	LOW DOSE 22-2444	HIGH DOSE 22-2442
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(49)
INFLAMMATION, FOCAL		2 (4%)	
LYMPHOCYTIC INFLAMMATORY INFILTR	2 (10%)	1 (2%)	3 (6%)
INFLAMMATION, NECROTIZING			1 (2%)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
GRANULOMA, NOS		1 (2%)	
NECROSIS, FOCAL		1 (2%)	
AMYLOIDOSIS			1 (2%)
METAMORPHOSIS FATTY		2 (4%)	1 (2%)
BASOPHILIC CYTO CHANGE			1 (2%)
HYPERPLASIA, FOCAL	1 (5%)		
*GALLBLADDER	(20)	(49)	(50)
DISTENTION			1 (2%)
#STOMACH	(20)	(49)	(48)
INFLAMMATION, ACUTE FOCAL			1 (2%)
PARASITISM			1 (2%)
#SMALL INTESTINE	(20)	(48)	(47)
ULCER, NOS		1 (2%)	
#INTESTINAL VILLUS	(20)	(48)	(47)
AMYLOIDOSIS			1 (2%)
#COLON	(20)	(49)	(47)
PARASITISM	2 (10%)	8 (16%)	11 (23%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(49)
CYST, NOS		1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)		4 (8%)
SCAR			1 (2%)
INFARCT, FOCAL		1 (2%)	1 (2%)
AMYLOIDOSIS			2 (4%)
ENDOCRINE SYSTEM			
#ADRENAL	(19)	(44)	(45)
AMYLOIDOSIS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONTINUED)

	CONTROL (VEH) 22-2446	LOW DOSE 22-2444	HIGH DOSE 22-2442
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(49)	(46)
CYST, NOS		1 (2%)	
AMYLOIDOSIS		1 (2%)	
#UTERUS/ENDOMETRIUM	(20)	(49)	(46)
CYST, NOS		3 (6%)	3 (7%)
HEMORRHAGIC CYST	1 (5%)		
INFLAMMATION, NOS	2 (10%)	1 (2%)	3 (7%)
HYPERPLASIA, NOS	2 (10%)	10 (20%)	13 (28%)
HYPERPLASIA, CYSTIC	5 (25%)	11 (22%)	9 (20%)
#OVARY	(19)	(37)	(37)
CYST, NOS	4 (21%)	5 (14%)	4 (11%)
PAROVARIAN CYST	2 (11%)	2 (5%)	2 (5%)
HEMORRHAGIC CYST			1 (3%)
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(49)
CORPORA AMYLACEA	3 (15%)	18 (37%)	11 (22%)
PSAMMOMA BODIES	1 (5%)	3 (6%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(49)	(50)
CONGESTION, NOS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONCLUDED)

	CONTROL (VEH) 22-2446	LOW DOSE 22-2444	HIGH DOSE 22-2442
BACTERIAL SEPTICEMIA		1 (2%)	
AMYLOIDOSIS			2 (4%)
LYMPHOCYTOSIS		1 (2%)	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	1	4
ANIMAL MISSING/NO NECROPSY		1	
AUTO/NECROPSY/HISTO PERF		1	4

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

Review of the Bioassay of 2-(Chloromethyl)Pyridine Hydrochloride*
for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

October 25, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, and State health officials. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 2-(Chloromethyl)Pyridine Hydrochloride for carcinogenicity.

The reviewer for the report on the bioassay of 2-(Chloromethyl) pyridine hydrochloride said that, under the conditions of test, the compound was not carcinogenic in treated rats or mice. He pointed out that the maximum tolerated dose may not have been tested in rats since there was no significant weight loss, mortality, or other signs of toxicity in the treatment group. There was no objection to a recommendation that the report on the bioassay of 2-(Chloromethyl) pyridine hydrochloride be accepted as written.

Clearinghouse Members Present:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
William Lijinsky, Frederick Cancer Research Center
Henry Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
Kenneth Wilcox, Michigan State Health Department

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

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